



Year: 2019

The secreted APP ectodomain sAPP⁺, but not sAPP⁻, protects neurons against A β oligomer-induced dendritic spine loss and increased tau phosphorylation

Tackenberg, Christian ; Nitsch, Roger M

Abstract: AIM The amyloid precursor protein (APP) is endoproteolytically processed to generate either the neurotoxic beta-amyloid peptide (A β) or the secreted ectodomain APP alpha (sAPP⁺). While neurotrophic properties of sAPP⁺ were suggested in several studies, it is still unclear if and how sAPP⁺ counteracts pathogenic effects of A β . Direct comparisons with sAPP⁻, produced in the A β -generating pathway, are missing in order to determine the role of sAPP⁺'s carboxyl-terminal end in its possible neuroprotective activity. **METHODS** Mouse neuronal primary cultures and hippocampal slices were treated with oligomeric A β . The effects on tau phosphorylation and dendritic spine densities were assessed by western blot and confocal imaging, respectively. Co-administration of either sAPP⁺ or sAPP⁻ was used to determine activity on A β -induced toxicity. **RESULTS/DISCUSSION** We found that oligomeric A β strongly increased AT8 and AT180 phosphorylation of tau and caused a loss of dendritic spines. sAPP⁺ completely abolished A β effects whereas sAPP⁻ had no such rescue activity. Interestingly, sAPP⁺ or sAPP⁻ alone neither affected tau phosphorylation nor dendritic spine numbers. Together, our data suggest that sAPP⁺ specifically protects neurons against A β -dependent toxicity supporting the strategy of activating γ -secretase-dependent endoproteolytic APP processing to increase sAPP⁺ shedding from the neuronal plasma membrane as a therapeutic intervention for the protection of dendritic spines and phospho-tau-dependent toxicity in Alzheimer's disease.

DOI: <https://doi.org/10.1186/s13041-019-0447-2>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-170437>

Journal Article

Published Version



The following work is licensed under a Creative Commons: Attribution 4.0 International (CC BY 4.0) License.

Originally published at:

Tackenberg, Christian; Nitsch, Roger M (2019). The secreted APP ectodomain sAPP⁺, but not sAPP⁻, protects neurons against A β oligomer-induced dendritic spine loss and increased tau phosphorylation. *Molecular Brain*, 12:27.

DOI: <https://doi.org/10.1186/s13041-019-0447-2>

MICRO REPORT

Open Access



The secreted APP ectodomain sAPP α , but not sAPP β , protects neurons against A β oligomer-induced dendritic spine loss and increased tau phosphorylation

Christian Tackenberg^{1,2*}  and Roger M. Nitsch^{1,2}

Abstract

Aim: The amyloid precursor protein (APP) is endoproteolytically processed to generate either the neurotoxic beta-amyloid peptide (A β) or the secreted ectodomain APP alpha (sAPP α). While neurotrophic properties of sAPP α were suggested in several studies, it is still unclear if and how sAPP α counteracts pathogenic effects of A β . Direct comparisons with sAPP β , produced in the A β -generating pathway, are missing in order to determine the role of sAPP α 's carbonyl-terminal end in its possible neuroprotective activity.

Methods: Mouse neuronal primary cultures and hippocampal slices were treated with oligomeric A β_{42} . The effects on tau phosphorylation and dendritic spine densities were assessed by western blot and confocal imaging, respectively. Co-administration of either sAPP α or sAPP β was used to determine activity on A β -induced toxicity.

Results/discussion: We found that oligomeric A β strongly increased AT8 and AT180 phosphorylation of tau and caused a loss of dendritic spines. SAPP α completely abolished A β effects whereas sAPP β had no such rescue activity. Interestingly, sAPP α or sAPP β alone neither affected tau phosphorylation nor dendritic spine numbers. Together, our data suggest that sAPP α specifically protects neurons against A β -dependent toxicity supporting the strategy of activating α -secretase-dependent endoproteolytic APP processing to increase sAPP α shedding from the neuronal plasma membrane as a therapeutic intervention for the protection of dendritic spines and phospho-tau-dependent toxicity in Alzheimer's disease.

Keywords: sAPP α , sAPP β , A β , Tau, Dendritic spines, Alzheimer's disease

Main text

The depositions of A β and hyperphosphorylated tau are the major hallmarks of Alzheimer's disease (AD). A β is produced by the amyloidogenic processing of APP. In this pathway, APP is cleaved by β -secretase (BACE-1) generating soluble APP β (sAPP β) and the membrane-bound fragment β -CTF. Further processing of β -CTF by γ -secretase produces the APP intracellular domain (AICD) and A β forms of various lengths. In the non-amyloidogenic pathway, A β generation is precluded by α -secretase, i.e. the metalloproteases ADAM10 and ADAM17, which cleave

within the A β sequence generating sAPP α and α -CTF. Subsequent γ -secretase cleavage releases AICD and the P3 fragment (reviewed in [1]).

Most AD-related studies focus on the detrimental effect of A β and therapeutic interventions aim in reducing A β levels. However, sAPP, especially sAPP α , is thought to have neurotrophic and neuroprotective properties [2] and its levels are strongly reduced in AD patients with one of two copies of APOE4, the main risk factor for AD. Interestingly, the levels of sAPP β were not different in AD patients compared to controls [3]. In contrast to sAPP α , sAPP β is not or much less potent in neurotrophic support [2]. In several studies on the beneficial or detrimental effects of sAPP α or sAPP β , the direct comparison of both is lacking. Here we aim to explore

* Correspondence: christian.tackenberg@irem.uzh.ch

¹Institute for Regenerative Medicine, University of Zurich, Schlieren, Switzerland

²Neuroscience Center Zurich, University of Zurich, Zurich, Switzerland



the neuroprotective properties of sAPP α on A β -induced neuronal dysfunction in direct comparison to sAPP β .

Oligomeric A β induces hyperphosphorylation of tau, which can cause toxicity downstream of A β [4]. To determine a potential effect of sAPP α on A β -induced tau phosphorylation primary neuronal cultures were transduced with neurotropic Sindbis virus to express the 441 amino acid isoform of human tau selectively in neurons [5].

Furthermore, cultures were treated with 500 nM oligomeric A β_{42} or scrambled A β_{42} (Additional file 1). Preparations of A β_{42} oligomers mainly consist of mono-, tri- and tetramers (Fig. 1a), which have been shown before to be cause neuronal dysfunctions [6]. As expected, treatment with oligomeric A β strongly increased tau phosphorylation at the AD-relevant epitopes AT8 and AT180 (Fig. 1b and c). The AT8 antibody detects phosphorylation at

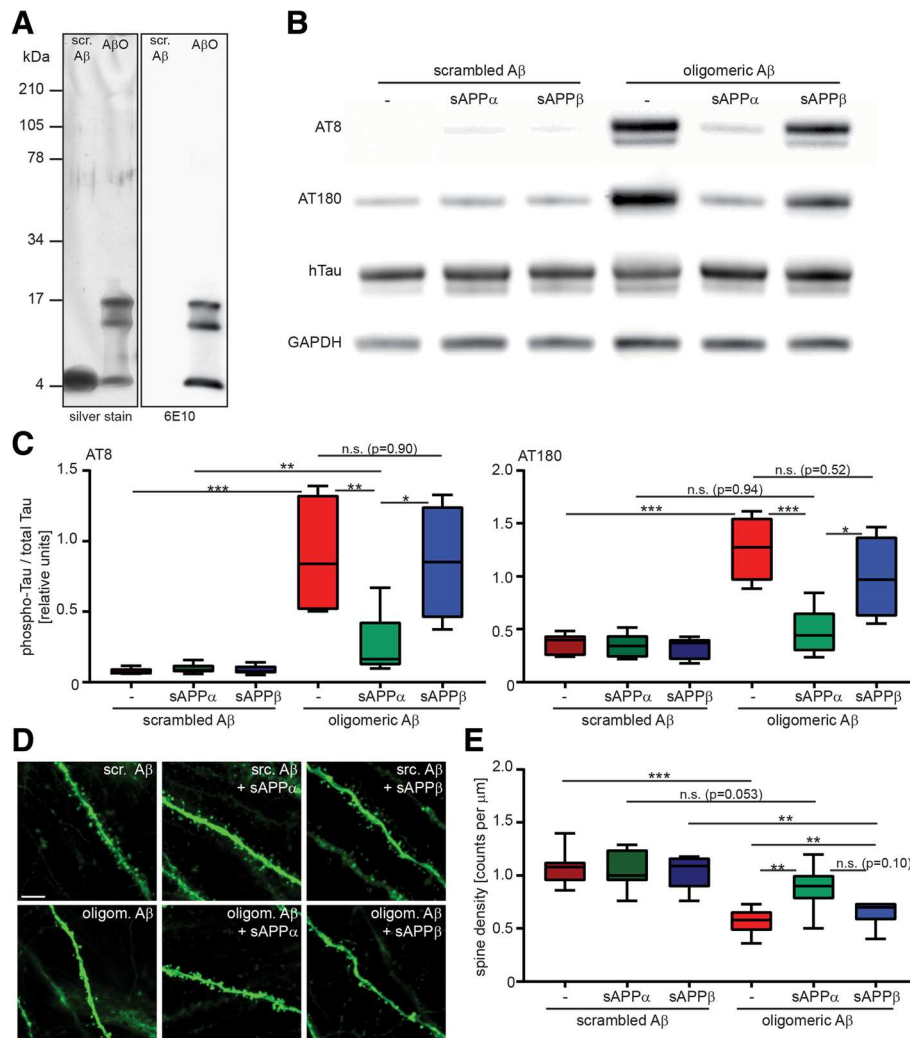


Fig. 1 Treatment with sAPP α but not sAPP β reduces A β -induced tau phosphorylation and dendritic spine loss. **a** Oligomeric A β preparations were characterized by SDS-PAGE followed by silver staining (left panel) or western blot using 6E10 antibody (right panel). Besides monomers, trimers and tetramers were the main A β species obtained by the used oligomerization protocol. **b** Primary neuronal cultures overexpressing human 441 tau were treated with 500 nM oligomeric A β or scrambled control and 400 ng/ml sAPP α or sAPP β . AT8 and AT180 phosphorylation was detected in cell lysates. **c** Quantification of western blots. A β oligomers increased AT8 and AT180 phosphorylation of tau which was prevented by 400 ng/ml sAPP α but not sAPP β . Statistical comparison between multiple groups was performed using one-way ANOVA with Tukey's test for multiple comparisons $n = 5$ (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). For important non-significant results, the exact p -values are displayed. **d** Representative images of CA1 apical dendritic segments of organotypic hippocampal slice cultures expressing eGFP. Slices were treated with 500 nM oligomeric A β or scrambled control and 400 ng/ml sAPP α or sAPP β . scale bar = 5 μ m **e** Analysis of dendritic spine density displayed as spine counts per μ m dendrite. Spine density was strongly reduced by A β treatment. sAPP α but not sAPP β diminished dendritic spine loss caused by A β . Statistical comparison between multiple groups was performed using one-way ANOVA with Tukey's test for multiple comparisons $n = 7-11$, Hippocampal slices were prepared from at least 3 different mice per condition. (**, $p < 0.01$; ***, $p < 0.001$). For important non-significant results, the exact p -values are displayed. Scr. A β : scrambled A β ; A β O: oligomeric A β

Ser202/Thr205, AT180 binds specifically to phosphorylated Thr231. Both epitopes are target of the main tau kinase, glycogen synthase kinase 3 beta (GSK-3 β) [7]. Co-treatment of cultures with 400 ng/ml recombinant sAPP α strongly reduced A β -induced tau phosphorylation. In contrast, treatment with sAPP β had no effect on tau phosphorylation in the presence of A β (Fig. 1b and c).

In addition, we analyzed whether sAPP α and sAPP β can modulate dendritic spine densities, as synapse loss strongly correlates with the degree of dementia in AD. To this end, we used organotypic slice cultures which were transduced with Sindbis virus to express enhanced GFP (EGFP). This method has been described before and allows morphological analysis of neuronal connectivity in slices [5, 6]. As previous studies showed that the expression of human tau in this system does not affect dendritic spine number or morphology, analysis of spines was performed in the absence of tau expression for this study [5, 8]. Images of dendritic segments in CA1 *stratum radiatum* were analyzed for dendritic spine density (Fig. 1d). CA1 *stratum radiatum* apical dendrites were chosen as they allow reliable imaging and evaluation due to the presence of long and straight dendritic segments. Further, A β affects different hippocampal regions, such as CA1 and CA3, to a similar extent [5]. We and others have shown that A β reduces the density of postsynaptic spines and alters their morphology in slice cultures (reviewed in [9]). Accordingly, treatment of slices with oligomeric A β but not scrambled A β strongly reduced dendritic spine numbers (Fig. 1e). We then determined if sAPP α or sAPP β may prevent spine loss. The presence of 400 ng/ml sAPP α completely abolished A β -induced spine loss while sAPP β -treated slices still displayed a significant spine reduction (Fig. 1e). To the best of our knowledge, this is the first report of a protective mechanism of sAPP α for A β -induced dendritic spine loss.

The CSF levels of sAPP in human patients reported in the literature strongly vary among different studies ranging from approx. 0,55 ng/ml and 0,25 ng/ml to 1800 ng/ml and 1600 ng/ml for sAPP α and sAPP β , respectively [3, 10]. Also, the ratios between sAPP α and sAPP β vary. For our analyses we used equimolar levels of sAPP α and sAPP β at concentrations within the range described in the literature. It is important to note that both, sAPP α and sAPP β alone, neither affect tau phosphorylation nor dendritic spine numbers. Thus, the effect of sAPP α represents a specific protective mechanism against A β -induced neuronal dysfunctions rather than a general neurotrophic effect.

sAPP α reduced A β -induced tau phosphorylation by increasing the expression of the A β -binding protein transthyretin (TTR) [11]. However, the A β concentrations used in that study were very high (50 μ M) and no comparison with sAPP β was performed. Since we used sAPP β as control we can clearly show that the protective property of sAPP α lies within the C-terminal part of the peptide.

Another study suggested that sAPP α reduces tau phosphorylation by GSK-3 β inhibition [12]. However, we did not observe a reduction in tau phosphorylation by treatment with sAPP α in the absence of A β oligomers. This implies that either sAPP α does not inhibit GSK-3 β in our model or that this inhibition only becomes noticeable after an activation of GSK-3 β by A β . Thus, it may be interesting to investigate the potential protective mechanism in our model in a future study in more detail.

Increasing sAPP α levels by activating α -secretase, specifically ADAM10, is of therapeutic potential for the treatment of neurodegenerative conditions including AD. Accordingly, mild overexpression of ADAM10 prevented amyloid plaque formation and hippocampal defects in transgenic AD mice [13]. However, ADAM10 is a widely distributed transmembrane protease and involved in shedding many different substrates. Clinical studies are required to determine whether therapeutic benefits of α -secretase activation would outweigh potential side effects (reviewed in [14]).

Another current strategy is the pharmacological reduction of A β production by inhibition of γ -secretase using γ -secretase inhibitors or modulators (GSIs, GSMs). Recently, it was shown that inhibition of γ -secretase activity activated a feedback loop leading to increased α -secretase processing and accelerated release of sAPP α [15]. Thus, GSIs may act via a dual protective mechanism, reduction of neurotoxic A β and elevation of neuroprotective sAPP α levels.

Taken together, restoration of sAPP α by increasing non-amyloidogenic processing can reduce A β pathology and therefore represents a valid therapeutic approach for AD (Additional file 1).

Additional file

Additional file 1: Soluble APP α but not soluble APP β protects against A β oligomer-induced dendritic spine loss and increased Tau phosphorylation. (PDF 143 kb)

Abbreviations

AD: Alzheimer's disease; ADDLs: A β -derived diffusible ligands; APP: Amyloid precursor protein; A β : Beta-amyloid peptide; A β O: Oligomeric A β ; GSIs: γ -secretase inhibitors; GSK-3 β : Glycogen synthase kinase 3 beta; sAPP: Soluble APP; Scr. A β : Scrambled A β ; TTR: Transthyretin

Acknowledgements

We thank Prof. Lawrence Rajendran (University of Zurich) for kindly providing recombinant sAPP.

Funding

CT was supported by the Deutsche Forschungsgemeinschaft (DFG, Ta762/1-1).

Availability of data and materials

The datasets used and/or analysed during this study are available from the corresponding author upon reasonable request.

Authors' contributions

CT and RMN conceived and designed the experiments. CT performed the experiments and wrote the manuscript. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

Animal experiments were performed in accordance with the guidelines of the veterinary office of the canton Zurich.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 1 February 2019 Accepted: 14 March 2019

Published online: 29 March 2019

References

- Haass C, Kaether C, Thinakaran G, Sisodia S. Trafficking and proteolytic processing of APP. *Cold Spring Harb Perspect Med*. 2012;2(5):a006270.
- Habib A, Sawmiller D, Tan J. Restoring soluble amyloid precursor protein alpha functions as a potential treatment for Alzheimer's disease. *J Neurosci Res*. 2017;95(4):973–91.
- Olsson A, Hoglund K, Sjogren M, Andreassen N, Minthon L, Lannfelt L, et al. Measurement of alpha- and beta-secretase cleaved amyloid precursor protein in cerebrospinal fluid from Alzheimer patients. *Exp Neurol*. 2003; 183(1):74–80.
- Blurton-Jones M, Laferla FM. Pathways by which Abeta facilitates tau pathology. *Curr Alzheimer Res*. 2006;3:437–48.
- Tackenberg C, Grinschgl S, Trutzel A, Santucci AC, Frey MC, Konietzko U, et al. NMDA receptor subunit composition determines beta-amyloid-induced neurodegeneration and synaptic loss. *Cell Death Dis*. 2013;4:e608.
- Birnbaum JH, Bali J, Rajendran L, Nitsch RM, Tackenberg C. Calcium flux-independent NMDA receptor activity is required for Abeta oligomer-induced synaptic loss. *Cell Death Dis*. 2015;6:e1791.
- Wang JZ, Xia YY, Grundke-Iqbal I, Iqbal K. Abnormal hyperphosphorylation of tau: sites, regulation, and molecular mechanism of neurofibrillary degeneration. *J Alzheimers Dis*. 2013;33(Suppl 1):S123–39.
- Tackenberg C, Brandt R. Divergent pathways mediate spine alterations and cell death induced by amyloid-beta, wild-type tau, and R406W tau. *J Neurosci*. 2009;29:14439–50.
- Tackenberg C, Ghori A, Thin BR. Stubby or mushroom: spine pathology in Alzheimer's. In: Lahiri DK, editor. *Advances in Alzheimer's research*, vol. 2. United Arab Emirates: Bentham Science Publishers; 2014. p. 375–94.
- Augustis K, Axelsson M, Portelius E, Brinkmalm G, Andreasson U, Gustavsson MK, et al. Cerebrospinal fluid biomarkers of beta-amyloid metabolism in multiple sclerosis. *Mult Scler*. 2013;19(5):543–52.
- Stein TD, Anders NJ, DeCarli C, Chan SL, Mattson MP, Johnson JA. Neutralization of transthyretin reverses the neuroprotective effects of secreted amyloid precursor protein (APP) in APPSW mice resulting in tau phosphorylation and loss of hippocampal neurons: support for the amyloid hypothesis. *J Neurosci*. 2004;24(35):7707–17.
- Deng J, Habib A, Obregon DF, Barger SW, Giunta B, Wang YJ, et al. Soluble amyloid precursor protein alpha inhibits tau phosphorylation through modulation of GSK3beta signaling pathway. *J Neurochem*. 2015;135(3):630–7.
- Postina R, Schroeder A, Dewachter I, Bohl J, Schmitt U, Kojro E, et al. A disintegrin-metalloproteinase prevents amyloid plaque formation and hippocampal defects in an Alzheimer disease mouse model. *J Clin Invest*. 2004;113(10):1456–64.
- Yuan XZ, Sun S, Tan CC, Yu JT, Tan L. The role of ADAM10 in Alzheimer's disease. *J Alzheimers Dis*. 2017;58(2):303–22.
- Siegenthaler B, Bali J, Rajendran L. γ -Secretase regulates the α -secretase cleavage of the Alzheimer's disease, amyloid precursor protein. *Matters*. 2016. <https://doi.org/10.19185/matters.201601000003>.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

